Viral Respiratory Tract Infections: Detection Now and in the Future

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• President Elect Critical and Point-of-Care Testing Division, American Association of Clinical Chemistry

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Disclosures:

- Dr. Kiechle has nothing to disclose.
I. Viral Respiratory Tract Infections
   A. Introduction
   B. Detection
      1. Traditional
      2. Multiplex PCR – based
   C. Workflow analysis
   D. Memorial Healthcare System: 1740 beds, 6 hospitals
Outline (cont.)

1. Volumes of RVP

2. Virogram
   a) All ages
   b) Age-related (peds (<18 years); adult >18 yrs)

3. Viral co-infections – age related
   a) MHS
   b) Literature review

E. Bacterial Co-infections

F. RVP Summary

G. Future: Hybrid bacterial/viral detection

H. References
Respiratory Viral Infections

- Respiratory infections account for ~4 million deaths per year, about half of which are due to viruses.
- Common viruses can cause serious respiratory infections.
- New viruses are also being identified:
  - Metapneumovirus (MPV)
  - Severe acute respiratory syndrome coronavirus (SARS-CoV)
  - Avian influenza viruses H5N1, H7N9
  - Coronavirus NL63 and HKU1
  - Human bocavirus
  - Middle East respiratory syndrome coronavirus (MERS-CoV)
Why Identify the Virus?

• Many viruses have similar initial symptoms
  o Some patients will quickly deteriorate, while others could be sent home to recuperate with reassurance
  o Different viruses may require different isolation practices; allows hospital to utilize infection control practices where patients are separated into wards by virus type

• Important to distinguish viral from bacterial causes
  o Avoid unnecessary antibiotics
  o Select specific antiviral agents, if available

• By utilizing epidemiologic data from lab, can prescribe appropriate prophylactic treatments (influenza and RSV) when necessary for at risk patients
The chart below represents the respiratory virus prevalence from all patients tested (Jun 2012 – Jan 2013). Those viruses with subtypes are collectively counted under the main virus name.

Why Identify the Virus?

- As new pathogens emerge, the ability to exclude known viruses may help to more rapidly recognize and identify the presence of a new pathogen

- Possible cost savings:
  - Shorter ER times for diagnosis/triage
  - Quicker access to treatment
  - Shorter hospital stays
  - Ability to “cohort” patients to prevent sick patients from catching a second virus
Traditional Identification of Viral Pathogens

• Direct fluorescent-antibody assay and culture
  o Time consuming (slow turn-around-time)
  o Labor intensive/require expertise to interpret
  o Require monoclonal antibodies for viruses (for rapid cell culture)
  o Virus must be viable

• Direct antigen testing
  o Quick results
  o Sensitivity and specificity vary widely, usually less sensitive than culture
  o Some are simple to use point-of-care tests
Background: Detection of Respiratory Viruses

- Traditional microbiology method **was** the gold standard of viral cultures
- Tube cultures and/or shell vial cultures
- Advantage of increased sensitivity versus the rapid antigen tests and DFA (Direct Fluorescence Antibody) assays
- Disadvantage of taking 1-14 days to rule as sample negative
  - Some viruses do not grow well or at all in cell culture
Molecular-Based Viral Identification

- PCR (DNA/RNA)-based assays are gaining popularity
  - Quicker turn-around-time
  - Increased sensitivity
  - Quick development for emerging pathogens (does not rely on development of monoclonal antibody)
  - Ability to multiplex
Respiratory Virus Panels

• Can multiplex relatively easily, with minimal increase in cost

• More readily identify co-infections

• Identify virus more quickly than ordering tests sequentially, particularly when there isn’t a prevalent virus “in season”

• Sometimes a new virus may “cross-react” with an existing panel virus, aiding in identification until a specific test is available

• Ability to exclude many viruses simultaneously
When should a viral panel be used vs. a single virus test?

Single Virus Test

• During epidemic when there is one (or few) major virus(es) circulating
• When a new/prevalent pathogen suspected is not on a panel, but has a specific test
• When demand for test is too high for throughput available with panel

Viral Panel

• When there isn’t a single prevalent virus
  o Follow CDC data
• In hospital setting when infection control measures must be implemented
• To rule out many viruses at once when a new virus is suspected
Significance of Positive Test

• Sensitive Assay: carriership vs. symptomatic infection
  o 2% and 6% of healthy adults positive for RHNV or Influenza A
  o RHNV detectable by PCR for 2 weeks after symptoms
  o Immunocompromised may shed RSV in absence of symptoms

• Large panel (15 or greater agents) offers additional diagnosing value
  o Negative result – more valid if many agents targeted
  o Clinical effects generated by one virus may be amplified by co-infection with another virus

RVP Time Summary

Assay A:
Extraction Time (1.25 hrs) + 5.23 + 2.22 = 8.7 hrs

Assay B:
Extraction Time (1.25 hrs) + 4.67 + 1.51 = 7.43 hrs

- A = 10 steps  B = 5 steps
- Decreased hands-on time
- Overall shorter assay (1.27 hrs shorter)
- Decreased manipulation of PCR products which means reduced risk for contamination
Annual RVP Volumes from 2009-2013

<table>
<thead>
<tr>
<th>Year</th>
<th>Volumes</th>
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<tbody>
<tr>
<td>2009</td>
<td>4206</td>
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<tr>
<td>2010</td>
<td>2876</td>
</tr>
<tr>
<td>2011</td>
<td>3355</td>
</tr>
<tr>
<td>2012</td>
<td>4052</td>
</tr>
<tr>
<td>2013</td>
<td>6225</td>
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## RVP: 20 Viral Targets

<table>
<thead>
<tr>
<th>Influenza A</th>
<th>Parainfluenza-4</th>
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<tbody>
<tr>
<td>Influenza A – H3 Subtype</td>
<td>Adenovirus B/E</td>
</tr>
<tr>
<td>Influenza A – H1 Subtype</td>
<td>Adenovirus C</td>
</tr>
<tr>
<td>Influenza A – 2009 H1N1</td>
<td>Human Metapneumovirus</td>
</tr>
<tr>
<td>Influenza B</td>
<td>Rhinovirus</td>
</tr>
<tr>
<td>RSV A</td>
<td>Coronavirus 229E</td>
</tr>
<tr>
<td>RSV B</td>
<td>Coronavirus NL63</td>
</tr>
<tr>
<td>Parainfluenza-1</td>
<td>Coronavirus HKU1</td>
</tr>
<tr>
<td>Parainfluenza-2</td>
<td>Coronavirus OC43</td>
</tr>
<tr>
<td>Parainfluenza-3</td>
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</table>
FluA3 – Influenza A-H3 subtype; FluA1 – Influenza A-H1 subtype; FluH1 – Influenza A-2009 H1N1 subtype; FluB – Influenza A/B; RSV – Respiratory Syncytial Virus; PIV – Parainfluenza Virus; ADNV – Adenovirus; HMPV – Human Metapneumovirus; RHNV – Rhinovirus; CoV - Coronavirus
FluA3 – Influenza A-H3 subtype; FluA1 – Influenza A-H1 subtype; FluH1 – Influenza A-2009 H1N1 subtype; FluB – Influenza A/B; RSV – Respiratory Syncytial Virus; PIV – Parainfluenza Virus; ADNV – Adenovirus; HMPV – Human Metapneumovirus; RHNV – Rhinovirus; CoV - Coronavirus
Patients With Any Positive Result
(Pediatrics versus Adults)
Patients with One Virus Detected (Pediatrics versus Adults)
Viral Co-Infections

- MHS
- Literature review
- Bacterial co-infections
- RVP Summary
- Future: hybrid bacterial/viral detection
Clinical Impact of Viral Co-Infections

Growing evidence for prevalence of viral co-infection and the impact on disease severity\textsuperscript{1-3}

- Viral co-infection prevalence
  - On average co-infection rates are 20-30%
  - Ranges vary by study cohort and viruses interrogated
  - RSV + HRV/hMPV most commonly cited

- Clinical Impact
  - Children with RSV + HRV increased LoS
  - Infants 3x more at risk PICU admission

- Financial Impact
  - Increase length of hospital stay
  - Increased morbidity and cost of care for PICU
  - Ineffective patient cohorting could increase hospital acquired co-infection in children

\textsuperscript{1} Mansbach (Arch Pedi 2012)
\textsuperscript{2} Paranhos-Baccala (JCV 2008)
\textsuperscript{3} Richard (J Ped Inf Dis 2008), Semple (JID 2005)
Patients With >1 Virus Detected

<table>
<thead>
<tr>
<th>Month</th>
<th>Peds</th>
<th>Adults</th>
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<td>1</td>
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<tr>
<td>Aug'13</td>
<td>16</td>
<td>1</td>
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<tr>
<td>Sep'13</td>
<td>27</td>
<td>6</td>
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<tr>
<td>Oct'13</td>
<td>38</td>
<td>11</td>
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Months: Jul'13, Aug'13, Sep'13, Oct'13
Number of Adult Co-Infections (July – Oct 2013)
Number of Pediatric Co-Infections (July – Oct 2013)

<table>
<thead>
<tr>
<th>Virus</th>
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<td>RHNV</td>
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<td>7</td>
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<td>6</td>
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<tr>
<td>RHNV</td>
<td>4</td>
</tr>
<tr>
<td>PIV4</td>
<td>3</td>
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<td>2</td>
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<tr>
<td>RHNV</td>
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<td>C229E</td>
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<tr>
<td>RHNV</td>
<td>2</td>
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<tr>
<td>RSVA1</td>
<td>2</td>
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<td>ADVC</td>
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<tr>
<td>ADVC</td>
<td>2</td>
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<td>PIV1</td>
<td>2</td>
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<td>RHNV</td>
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<tr>
<td>FLUBP</td>
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<td>FLUH1</td>
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<td>HMPV</td>
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### Number of Pediatric Co-Infections (July – Oct 2013) continued

<table>
<thead>
<tr>
<th>PIV3</th>
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<th>RHNV</th>
<th>ADVB</th>
<th>CNL63</th>
<th>ADVC</th>
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<th>ADVB</th>
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Number of Pediatric Co-Infections (July – Oct 2013) continued

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<td><strong>RSVB1</strong></td>
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<td><strong>RSVA1</strong></td>
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<td><strong>FLUH1</strong></td>
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<td><strong>ADVE</strong></td>
<td><strong>PIV1</strong></td>
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<td><strong>RSVA1</strong></td>
<td><strong>FLUBP</strong></td>
<td><strong>FLUH1</strong></td>
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<td><strong>RSVA1</strong></td>
<td><strong>RHNV</strong></td>
<td><strong>ADVE</strong></td>
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<td><strong>FLUBP</strong></td>
<td><strong>FLUH1</strong></td>
<td><strong>PIVB1</strong></td>
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<td><strong>RHNV</strong></td>
<td><strong>ADVE</strong></td>
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<td><strong>RSVA1</strong></td>
<td><strong>FLUBP</strong></td>
<td><strong>FLUH1</strong></td>
<td><strong>PIVB1</strong></td>
<td><strong>RSVA1</strong></td>
<td><strong>RHNV</strong></td>
<td><strong>ADVE</strong></td>
<td><strong>PIV1</strong></td>
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### Number of Pediatric Co-Infections (July – Oct 2013) continued

<table>
<thead>
<tr>
<th>C229E</th>
<th>COC43</th>
<th>ADVE</th>
<th>RHNV</th>
<th>ADVE</th>
<th>ADVC</th>
<th>RHNV</th>
<th>FLUBP</th>
<th>COC43</th>
<th>C229E</th>
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<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>RSVB1</td>
<td>ADVB</td>
<td>COC43</td>
<td>ADVC</td>
<td>FLUBP</td>
<td>FLUH1</td>
<td>PIV1</td>
<td>PIV4</td>
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</table>
Age Distribution of Specimen Requests and the Virus Detection (acute respiratory tract infections)

<table>
<thead>
<tr>
<th>Age Group</th>
<th>No. of Total Specimens</th>
<th>No.(%) of virus-isolated specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-9 years</td>
<td>4212</td>
<td>3065 (91.5%)</td>
</tr>
<tr>
<td>10-19 years</td>
<td>188</td>
<td>77 (2.3%)</td>
</tr>
<tr>
<td>20-39 years</td>
<td>131</td>
<td>28 (0.84%)</td>
</tr>
<tr>
<td>40-59 years</td>
<td>218</td>
<td>4 (1.34%)</td>
</tr>
<tr>
<td>60-79 years</td>
<td>466</td>
<td>112 (3.3%)</td>
</tr>
<tr>
<td>80-99 years</td>
<td>103</td>
<td>23 (0.7%)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>5318</td>
<td>3,350 (100%)</td>
</tr>
</tbody>
</table>

# Distribution of Infection Types

<table>
<thead>
<tr>
<th>Infection Type</th>
<th>No. (%) of Infected Specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single infection</td>
<td>2717 (81.1%)</td>
</tr>
<tr>
<td>Double infection</td>
<td>572 (17.1%)</td>
</tr>
<tr>
<td>Triple infection</td>
<td>60 (1.8%)</td>
</tr>
<tr>
<td>Quadruple infection</td>
<td>1 (0.03%)</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>3,250 (100%)</strong></td>
</tr>
</tbody>
</table>

# Lower Respiratory Tract Infections – Hospitalized Children (Norway)

<table>
<thead>
<tr>
<th>Virus</th>
<th>Norway</th>
<th>Korea</th>
<th>Florida</th>
</tr>
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<tbody>
<tr>
<td>RSV</td>
<td>40.3%</td>
<td>-</td>
<td>32%</td>
</tr>
<tr>
<td>CoV OC43</td>
<td>73%</td>
<td>48%</td>
<td>13%</td>
</tr>
<tr>
<td>CoV NL63</td>
<td>40%</td>
<td>NT</td>
<td>17%</td>
</tr>
<tr>
<td>CoV 229E</td>
<td>0</td>
<td>38.2%</td>
<td>22%</td>
</tr>
<tr>
<td>CoV HKU1</td>
<td>0</td>
<td>NT</td>
<td>0</td>
</tr>
</tbody>
</table>

Norway: CoV = shorter fever period and shorter LOS compared to RSV
NT = not tested

---


Bacterial Co-Infections

- Viral infection leads to increased susceptibility to bacterial co-infections
  - RSV
  - Secondary bacterial pneumonia caused fatalities in 1918-1919 flu pandemic
Bacterial Co-Infections: Etiology

1. Altered physical barriers
   - Damage to lung epithelia increasing bacterial entry
   - Flu virus neuraminitidase thins mucus and exposes epithelial cell receptors

2. Altered immune system
   - Viral infection allows greater bacterial infections
   - Flu infection inhibits neutrophilia

Respiratory Panel Considerations

- Negative results do not exclude the possibility of infection with a respiratory virus as the virus could be below the assay limit of detection.
- Positive results do not exclude the possibility of co-infection with other viruses or bacteria, or concurrent underlying pulmonary pathology.
Respiratory Panel Considerations

- Specificity and sensitivity for each virus, throughput, and turn-around-time vary greatly among commercially available panels.

- Unique characteristics of the patient population being treated must be considered in selecting a panel:
  - What viruses are my patients at risk for contracting?
  - How timely does the result need to be received to clinically impact patient care?

- When multiple testing options are available, good communication between the laboratory and treating physicians is essential for optimal patient care.
Future: Hybrid approach to Viral / Bacterial Respiratory Tract Infections

1. ID using colony on agar plate
   - MALDI-TOF (matrix-assisted laser desorption ionization-time of flight)
   - Whole genome sequence using NGS

3. RVP combined with multiplex assays to detect either groups of Gram-positive or Gram-negative bacteria

4. POCT for rapid diagnosis of viral/bacterial RTIs based on multiplex molecular microfluidic method – gap-fill TAT issues with central lab-based PCR
References


Acknowledgments

• Rodney Arcenas, PhD, Molecular Diagnostics Lab Director
• Paul A. Malek, MD, PCSB Leader
• CAP Staff
Upcoming Free Webinars

- Prenatal Screening for Down Syndrome: Past, Present and Emerging Practices
  - March 20 at 11 am Central
  - Presented by Glenn Palomaki, PhD

- Common Cancer Genes Used by NGS Pathologists Early Adopters Panels
  - May 7 at 11 am Central
  - Presented by Mary M. Zutter, MD, FCAP

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# CAP Learning – Molecular and Diagnosis of Respiratory Viruses

## Course Learning Objectives

<table>
<thead>
<tr>
<th>Course</th>
<th>Learning Objectives</th>
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| 2012 CPIP Case 08 - Respiratory Viruses  
CME/SAM – 1.25 | As a result of participating in this activity, you will be able to:  
• Discuss specimen collection for respiratory viral testing.  
• Explain the limitations of rapid antigen detection testing for influenza.  
• Review the concepts of antigenic shift and drift.  
• Recognize currently available FDA cleared molecular tests for respiratory viral testing. |
The CAP Learning Portal includes content and tools designed to support the learning needs of pathologists. A user must login to cap.org in order to access the portal. In the portal, you will find:

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- **Now Available:**
  - Emerging Concepts in the Diagnosis of Respiratory Viruses (NEW)
  - Emerging Concepts in Molecular Testing in Breast Cancer (NEW)
  - Emerging Concepts in the Workup of Colorectal Cancer
  - Emerging Concepts in Therapeutic Guidance for Metastatic Melanoma
  - Emerging Concepts in the Diagnosis and Workup of Thyroid Cancer
  - Emerging Concepts in Colorectal Cancer Hereditary Non-Polyposis Cancer (Lynch Syndrome)
  - Emerging Concepts in the Workup of Polycythemia and Thrombocytethemia: JAK2

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Molecular Pathology (single gene, small panel)
Genomic Analysis (large panels, exome, genome)
Digital Pathology
In Vivo Microscopy

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- CAP member pathologists’ partner with gynecologists, radiologists and other medical professionals to lead See, Test & Treat programs in hospitals, clinics and other facilities

- Women learn the importance of preventive care through annual exams, a Pap test, Mammogram and a healthy lifestyle

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THANK YOU!

Thank you for attending our webinar “Viral Respiratory Tract Infections: Detection Now and in the Future” by Frederick L Kiechle MD, PhD, FCAP

For comments about this webinar or suggestions for upcoming webinars, please contact Jill Kaufman, PhD, Director of Personalized Health Care at jkaufma@cap.org

NOTE: There is no CME/CE credit available for today’s free webinar.